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Analysis of Fixed Retina Samples

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Analysis: Jeri Timlin (7-27-14)

U.S. DEPARTMENT OF ENERGY | NNSA National Nuclear Security Administration

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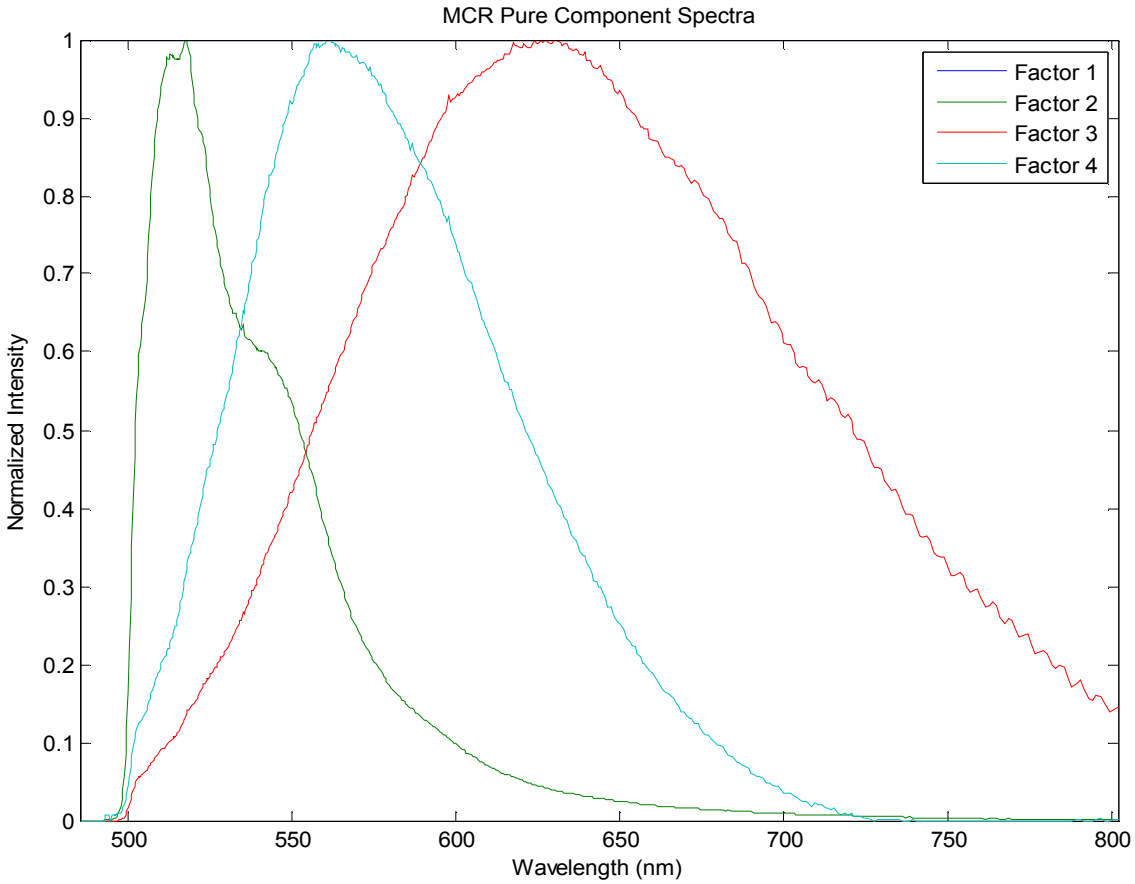
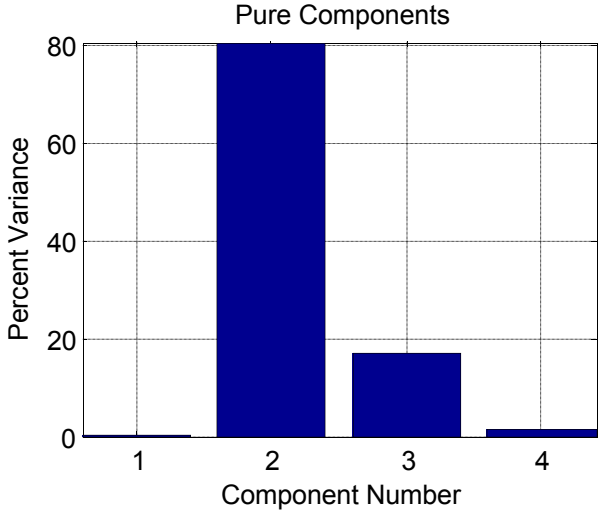
Imaging/Analyiss Details

- 488 nm excitation, OD 1 used for GFP expressing samples, OD 0 used for non-GFP samples
- 20x objective, 100 x 100 um field of view
- Spatial resolution: approximately 500nm in X&Y and 1.0 um in Z
- Analysis: MCR analysis (ImageMCR) was run on a composite set of all the images taken (compressed 4x) to identify pure components. Model was clean and easy to obtain. Then CLS was performed on each individual image at full spatial resolution (RAPID) to obtain final concentrations of each component.

MCR Analysis





This is the percent variance averaged across all of the data – this varies from image to image, depending on the image.

Questions: Spectrally I would interpret Factor 4 to be DAPI (we collect only the tail of it), however if Dapi stains the cell nuclei this is not correct. Neither factor 3 or 4 really look right for DAPI .

Summary

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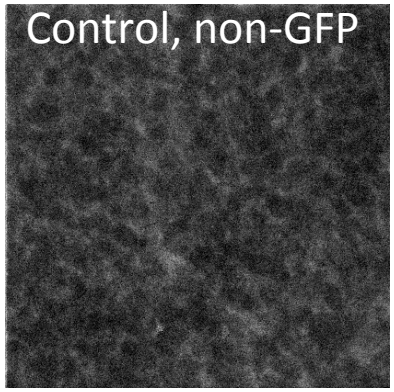
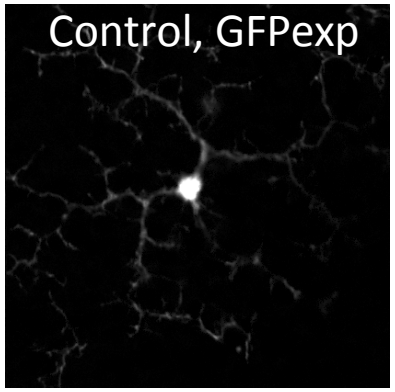
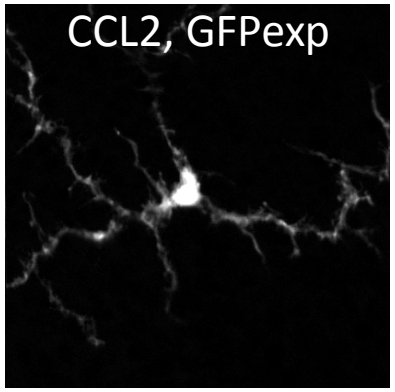
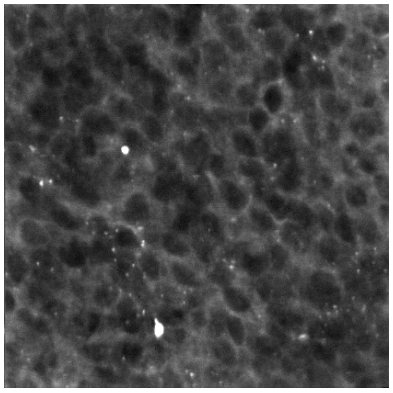
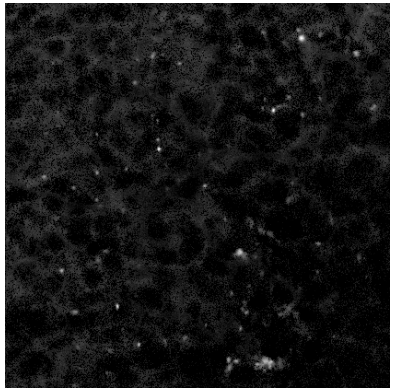
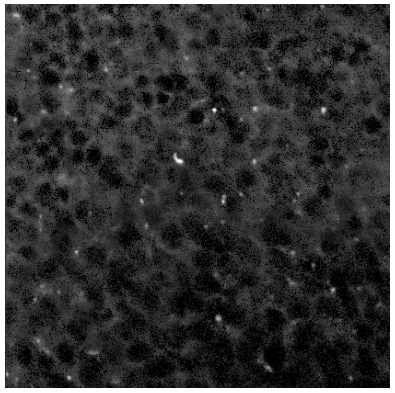
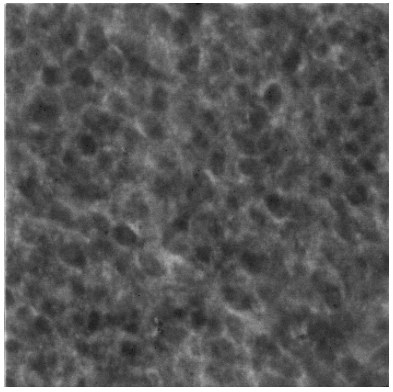
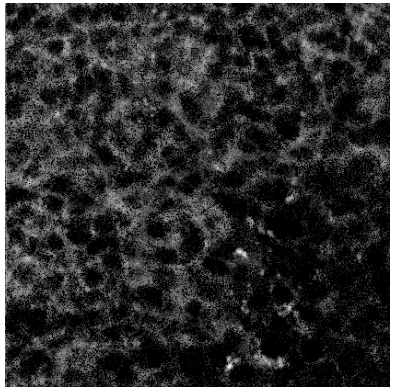
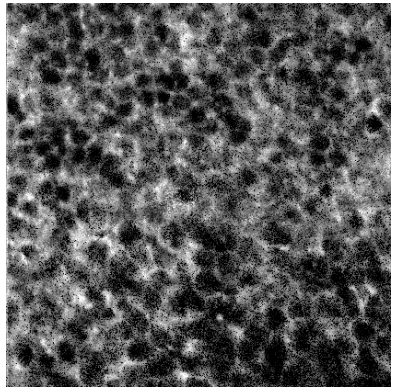
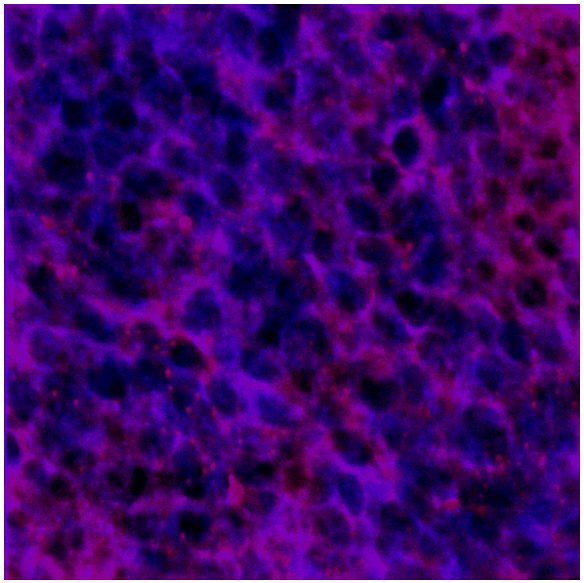
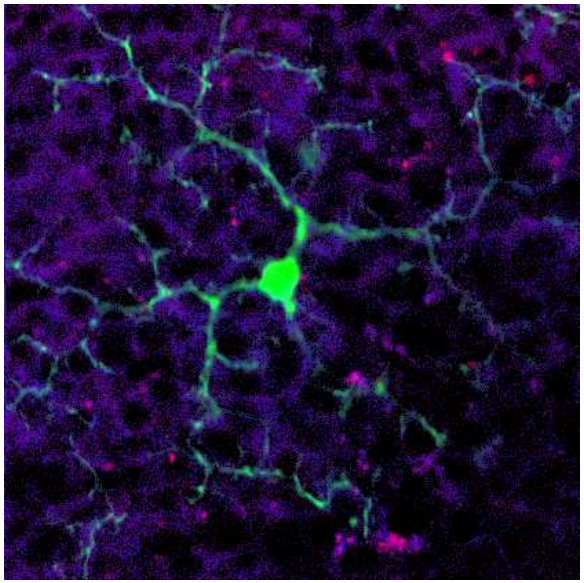
	Control, non-GFP		Control, GFPexp	CCL2, GFPexp	
GFP		Min = 0 Max = 1000 If this were on the same scale as the others this would be black, model is just fitting noise.			Min = 0 Max = 31000
AF red		Min = 200 Max = 16000			Min = 200 Max = 6000
AF teal		Min = 500 Max = 12000			Min = 500 Max = 2500
	C5781_6_control_1_4.dat		CX3_CR1_GFP_Control_1_9.dat	CX3_CR1_GFP_INJ_CCL2_1_9.dat	

Sample RGB Images



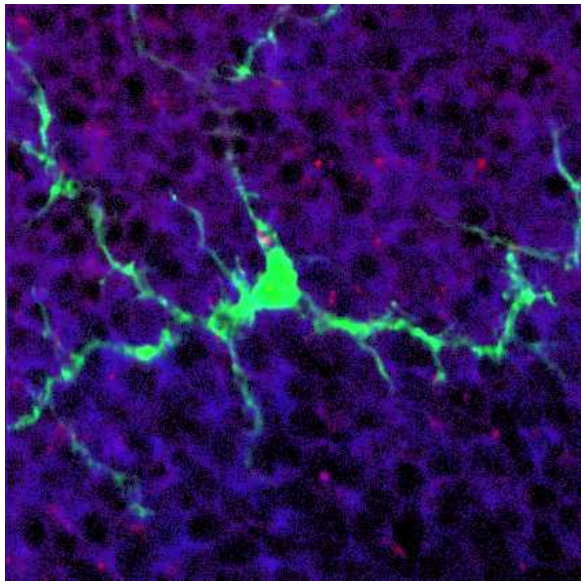
Control/No GFP

C5781_6_control_1_4.dat



Control/GFP exp

CX3_CR1_GFP_Control_1_9.dat

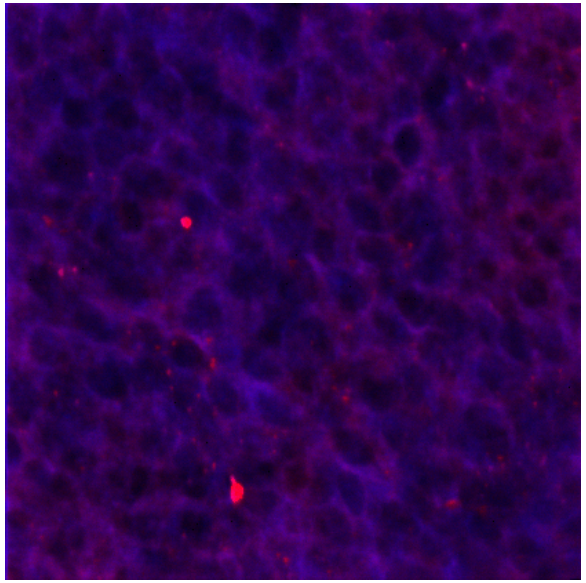


CCL2/GFP exp

CX3_CR1_GFP_INJ_CCL2_1_9.dat

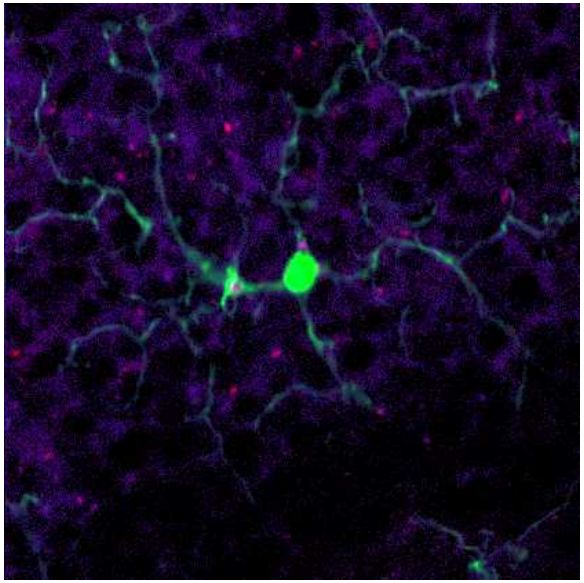
Note: Colorscale is NOT the same for each image.

Sample RGB Images



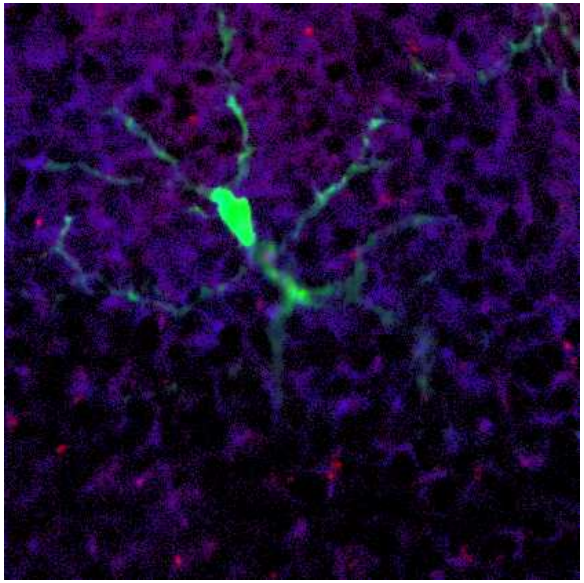
Control/No GFP

C5781_6_control_1_3.dat



Control/GFP exp

CX3_CR1_GFP_Control_1_5.dat



CCL2/GFP exp

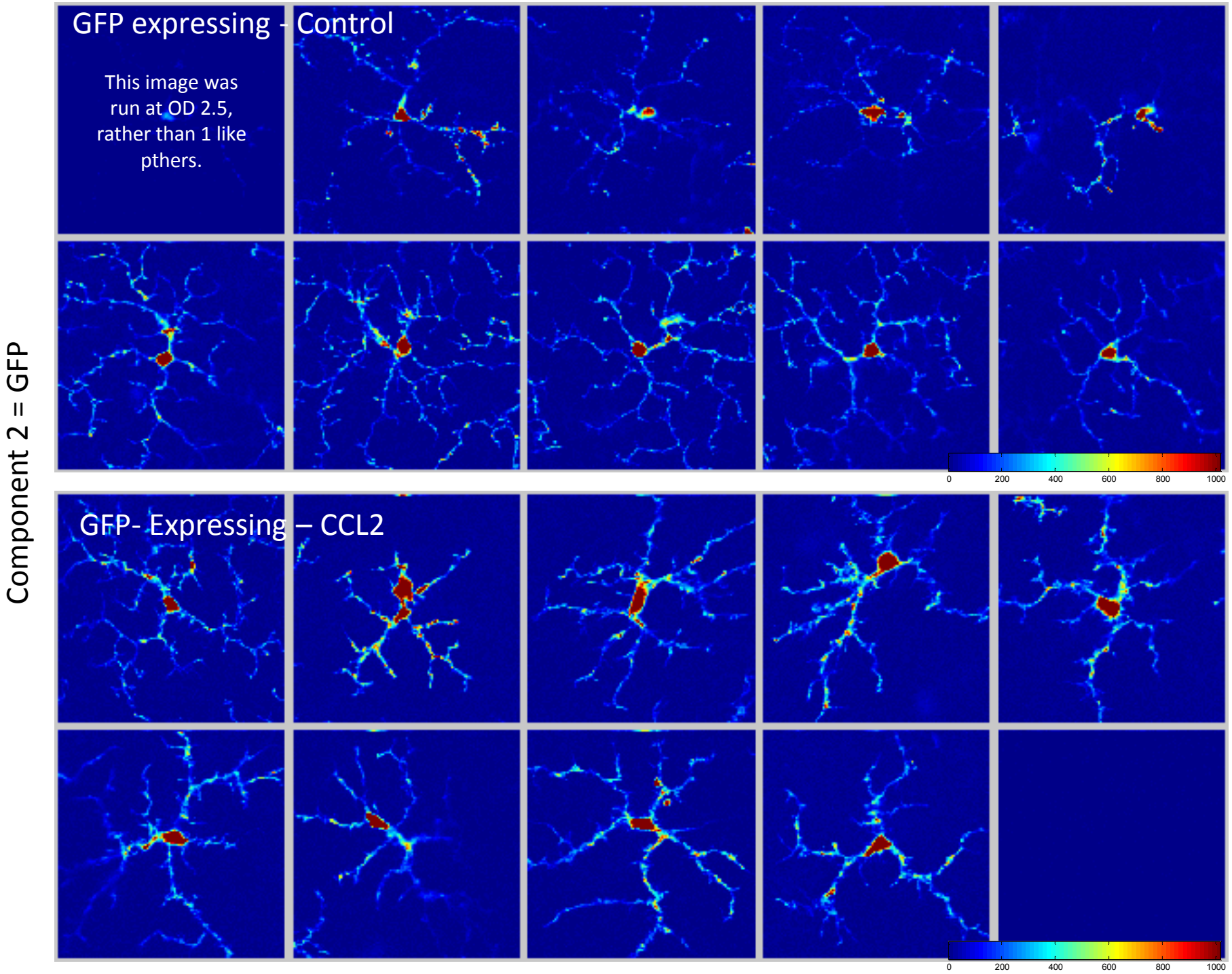
CX3_CR1_GFP_INJ_CCL2_1_5.dat

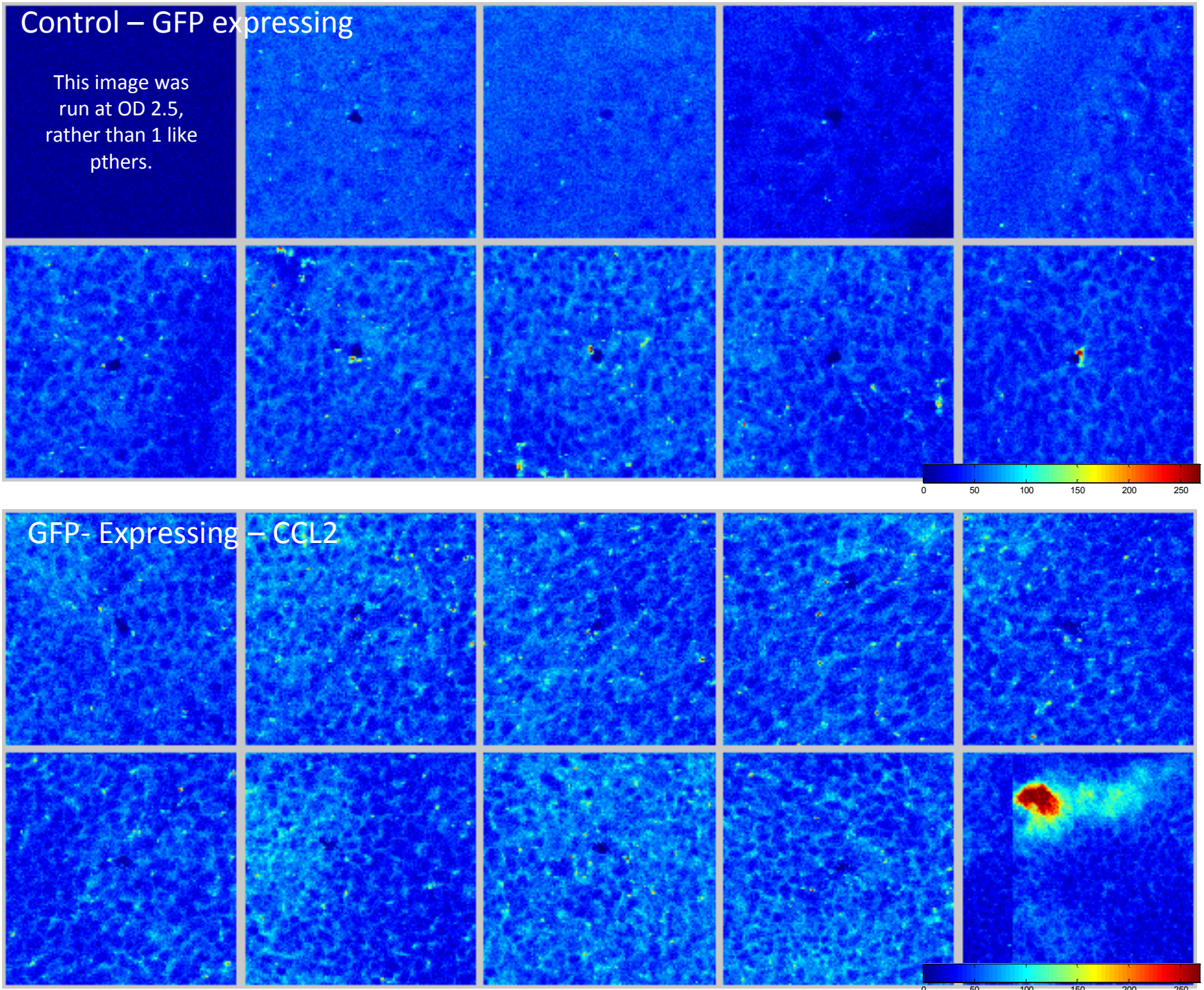
Note: Colorscale is NOT the same for each image.

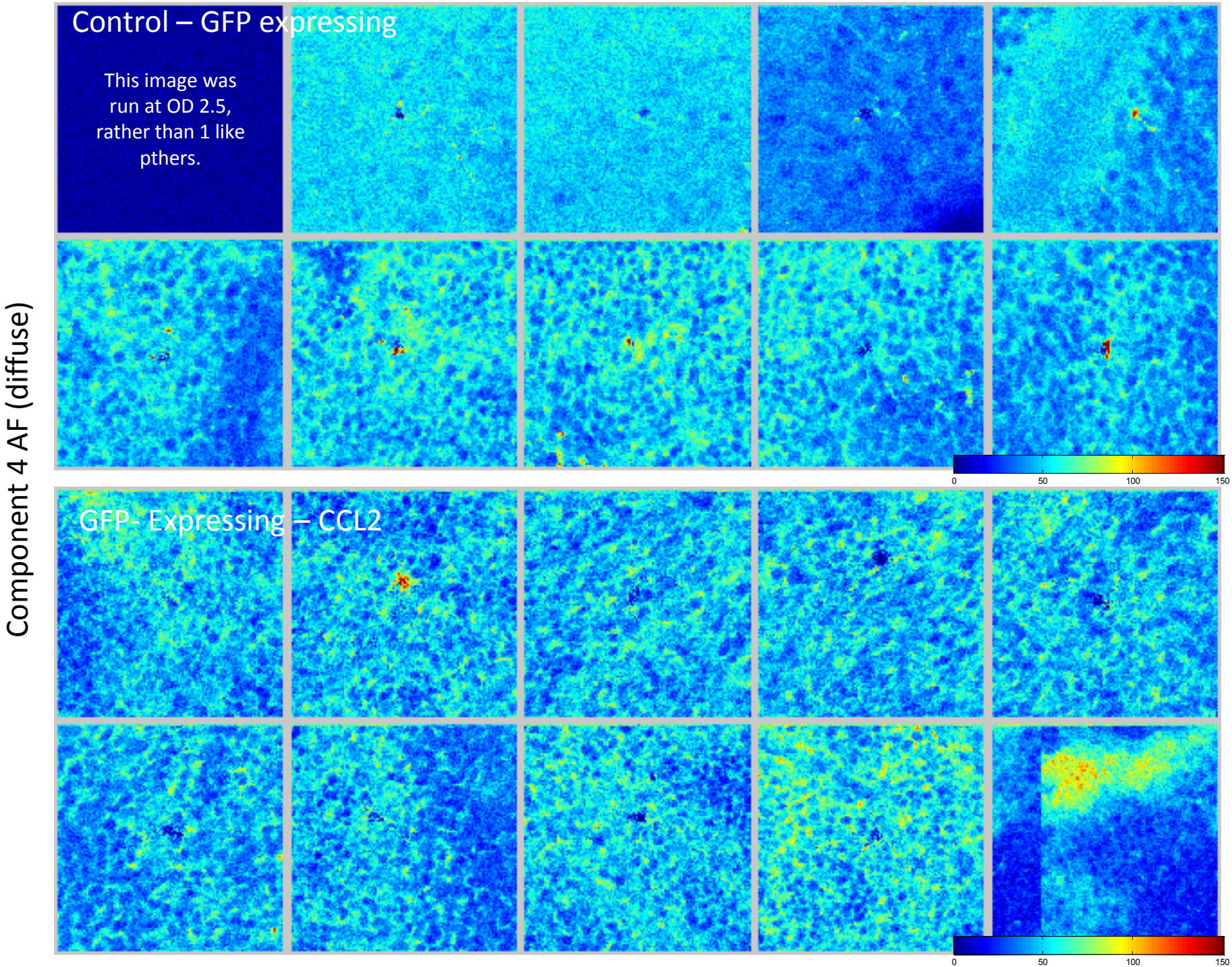
These next slides show the individual components for all images of CC2 treated and untreated retinas

All images on the following slides are 100um x 100um and all images within a slide are plotted on the same scale to facilitate direct comparison and assessment of morphological differences.

Note: I don’t know why the bottom right image is messed up. The original looks fine. My guess is something happened in the preprocessing since we are right at the edge of the memory available.


GFP expressing - Control
This image was run at OD 2.5, rather than 1 like pthers.
0
200
400
600
800
1000
GFP- Expressing – CCL2
0
200
400
600
800
1000
Component 2 = GFP


Control – GFP expressing
This image was run at OD 2.5, rather than 1 like pthers.
GFP- Expressing – CCL2
Component 3 AF (puncta)
0
50
100
150
200
250


Control – GFP expressing
This image was run at OD 2.5, rather than 1 like pthers.
GFP- Expressing – CCL2
Component 4 AF (diffuse)
0
50
100
150
0
50
100
150

Summary

- What are the identities of the two AF components? Is one a dye?
- No drastic differences (intensity, morphology, or spectral shift) between control and CCL2 treated retinas in any component (GFP or AF) in the GFP expressing mice. Subtle differences are possible…
  - Maybe a slight hint of the processes being retracted in the CCL2 treated images
  - AF (blue) is possibly more intense in the CCL2 treated mice
- There's an intensity difference in the non-GFP data AF components compared to the GFP expressing AF components, however this is due to different excitation intensities during the scan.